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6 INVESTIGATION OF THE BIOLOGICAL EFFECTS OF
PULSED ELECTRICAL FIELDS

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I. INTRODUCTION

Research conducted during the contracting period has involved a continuation of an investigation of the in vivo effects of exposure of Dutch rabbits to electromagnetic pulsed (EMP) fields and an in vitro study of the effects of transient electrical and electromagnetic fields on biomembranes. Phenomenological studies of the effects of EMP exposure of Dutch rabbits have been undertaken in an attempt to characterize the nature of the alterations induced by such fields, whereas the biomembrane studies have been directed toward a mechanistic understanding of field-induced alterations in biological model systems.

Previous studies of the in vivo effects of EMP exposure suggested that the limited exposure conditions employed did not result in statistically significant alterations in a number of biological endpoints, including serum chemistry, serum triglyceride levels, serum enzymes, and drug-induced sleeping time. In view of the fact that these dependent variables have been found to be significantly affected by low level thermal stress induced either by microwave exposure or elevated environmental temperatures, it may be concluded that EMP does not induce similar stress effects in the Dutch rabbit. This result would be anticipated on both theoretical and experimental bases since the EMP exposure conditions were such that the average field power dissipated in the experimental subjects was such that no detectable temperature rise was involved. Although the results of studies of EMP effects on the Dutch rabbit were not statistically significant, there appeared to be consistent low-level elevation in serum enzyme levels. Elevations in serum levels of intracellular enzymes are associated either with cell destruction or alteration in cell membrane permeability. In the absence of other physiological responses indicative of EMP-induced cell death, it has been concluded that

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the increased serum enzyme levels may be related to altered cell membrane permeability. The in vitro studies have thus been directed toward the determination of the conditions for field-induced alterations in biomembrane permeability. The membrane cation permeability is a more sensitive endpoint than enzyme permeability, consequently the effects of electrical and electromagnetic fields on the potassium (K^+) and sodium (Na^+) fluxes have been investigated under various exposure conditions and field strength and pulse duration thresholds have been determined for erythrocytes.] Techniques have been developed for the study of cation fluxes and enzyme permeation of biomembranes of lymphocytes and platelets to provide data on other cell types and sizes. Theoretical analyses have been performed to determine the effect of variation of cell size and shape on the induced transmembrane potential which will permit the correlation of effects in various cells to the field parameters. A theoretical comparison has also been made of the relative magnitude of the field induced in a biomembrane by inductive and conductive fields in order to provide a basis for the comparison of the in vivo inductive field exposures with the in vitro conductive field exposures.

II. EFFECTS OF PULSED ELECTRICAL FIELDS ON ERYTHROCYTE MEMBRANES

There have been a number of reports concerned with the effects of electric field pulses on cell membrane permeability, particularly erythrocytes¹⁻¹⁰. In most instances, cells were exposed in saline suspensions to a single conductive electric field pulse. It was observed that above a critical electric field strength the permeability of the erythrocyte membrane to sodium and potassium was altered, and at higher field strengths or longer pulse durations hemoglobin leakage occurs. Electric field-induced permeability changes have been used for the production of erythrocyte ghosts³, the loading of cells with enzymes⁴, and for studying the stability of cell membranes in the presence of various chemical substances^{5,6}. The work reported here is concerned with the cumulative effect of multiple pulses of varying duration to provide information on the processes involved in field-induced membrane alterations.

Erythrocytes from human, dog, and rabbit donors were washed and resuspended in potassium-free, buffered, normal saline immediately after the samples were obtained by venipuncture. The cell suspensions were placed between platinum electrodes in a lucite exposure chamber to which high-voltage pulses were applied. The results of typical exposures of human erythrocyte suspensions are shown in Figures 1(A) and 1(B). Fig. 1(A) shows the effects of exposure to pulses with a peak pulse amplitude of 4.8 KV/cm and an exponential decay time constant, τ of 6 μ s. The figure shows the percentage change in extracellular K^+ , osmotic fragility, and hemolysis, relative to erythrocytes osmotically lysed in distilled water as a function of the number of applied pulses. Figure 1(B) summarizes the results of exposure to 0.75 μ sec pulses of the same amplitude. For $\tau = 6\mu$ s, potassium release was complete after a single pulse, while for $\tau = 0.75\mu$ s, 218 pulses were required to produce the same amount of intracellular

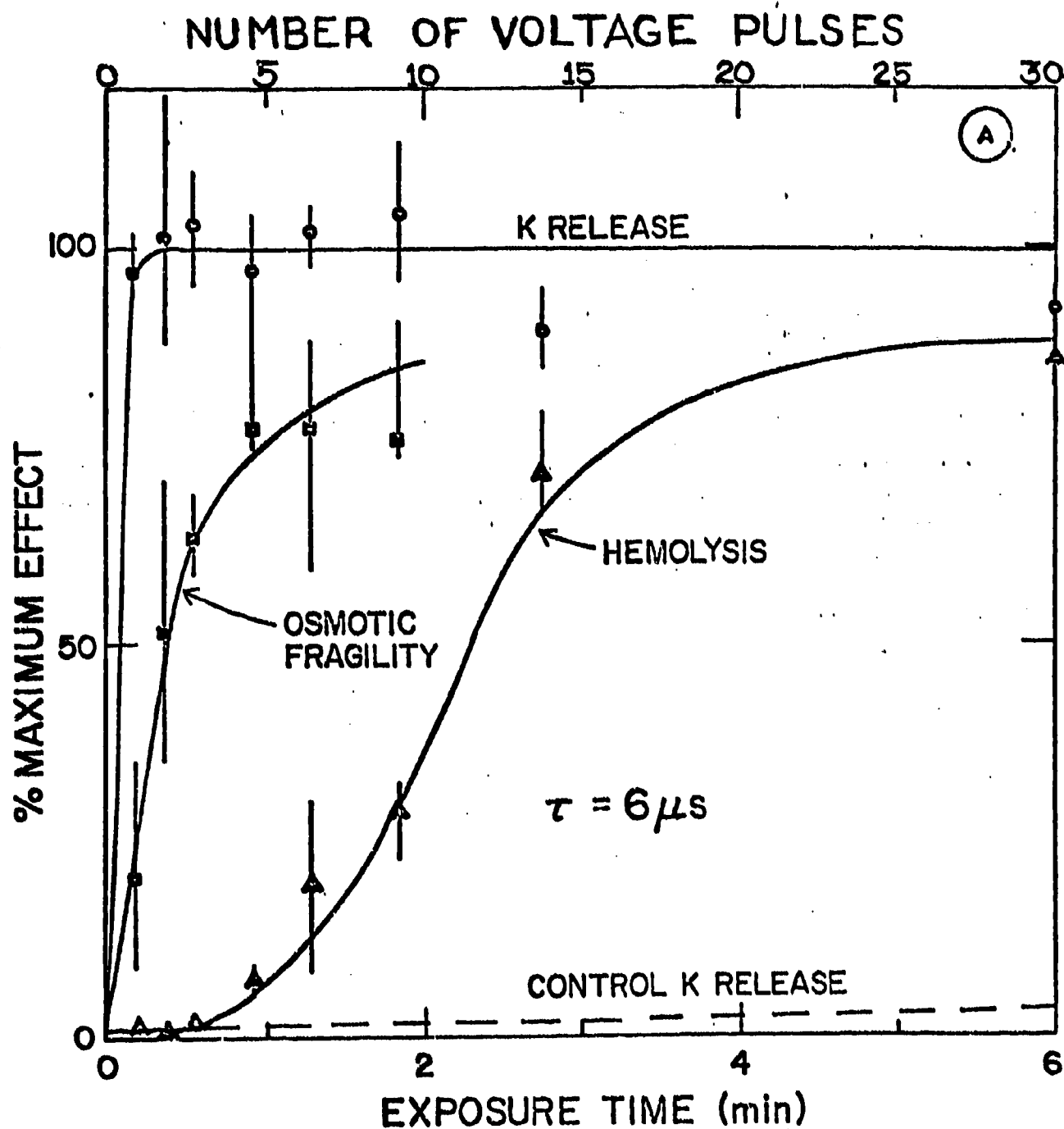


Fig. 1(A) Results of exposure of human erythrocyte suspensions to electric field pulses: (A) pulse-amplitude 4.8 KV/cm and decay time constant 6 μ sec and (B) pulse-amplitude 4.8 KV/cm and decay time constant 0.75 μ sec. The time between pulses was 11 sec for 6- μ sec pulses and 1.4 sec for the 0.75- μ sec pulses. Each data point represents the average of two exposures of erythrocytes from the same donor, with each quantity determined three times for each exposed sample. The error bars show the extremes of the six values thus obtained.

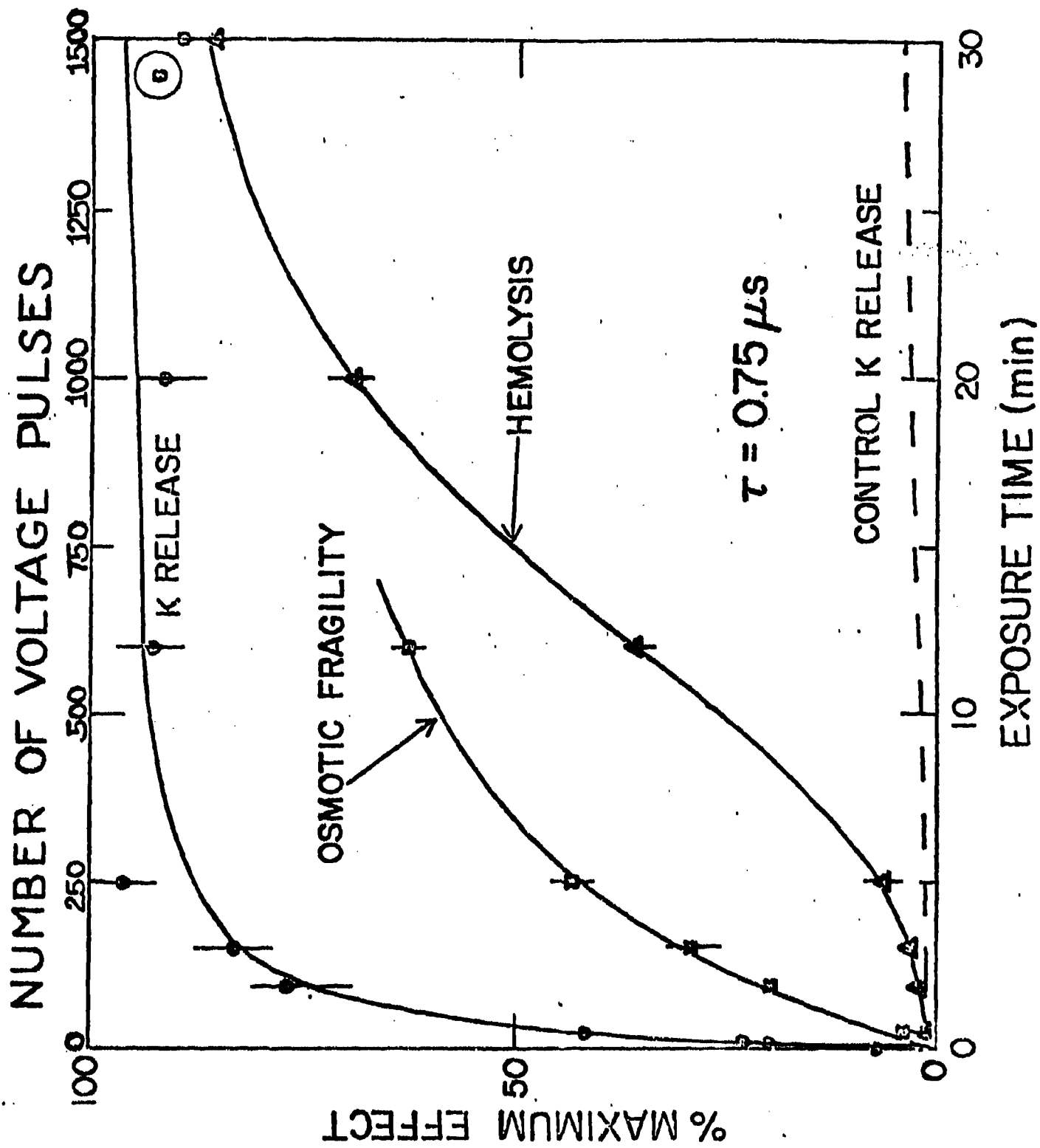


Figure 1(B)

~~K⁺ release. [REDACTED]~~

~~erythrocytes require larger numbers of pulses for a given level of effect.~~

~~membrane K⁺ release. Hemolysis is not evident until K⁺ release is~~

~~complete.~~ In spite of the fact that the average power to which the cell suspensions were exposed was the same for the 6 μ S pulse and 0.75 μ S pulses, the number of exposures to produce a given effect with the 0.75 μ S pulse was approximately 10 times that required with a 6 μ S pulse. When the time constant is further decreased to 0.43 μ S, at a field strength of 4.8 KV/cm, only 45% K⁺ potassium release was produced by an exposure to 4,000 pulses and no increase in osmotic fragility or hemoglobin release was observed. These data indicate that at this field strength there is a minimum pulse duration, below which no detectable effect on membrane structure is produced. ~~[REDACTED]~~

~~minimum pulse amplitude of approximately 2 KV/cm is necessary to produce~~

~~maximum release from normal, freshly prepared human erythrocytes.~~ The results shown in Figure 2 are similar to those obtained by other investigators using single pulses of longer duration ^{1,2,5,7}. Results obtained with rabbit and dog erythrocytes were qualitatively similar to those for human erythrocytes, but for given exposure conditions, all three dependent variables were affected to a greater extent in human erythrocytes indicating a heightened sensitivity to the effects of exposure to pulsed electrical fields.

None of these effects can be attributed to heating of the cell suspensions. All exposures were at 24°C, and the mean temperature increase during exposure was <0.3°C. Control suspensions of erythrocytes incubated at 37°C for 3 hours showed maximum increases of 6% for intracellular potassium release and 3% for hemoglobin release or for osmotic fragility.

*also duration
is critical
parameter.
longer pulse - more
effective.*

*2,000 u/c-
= 20 u/m*

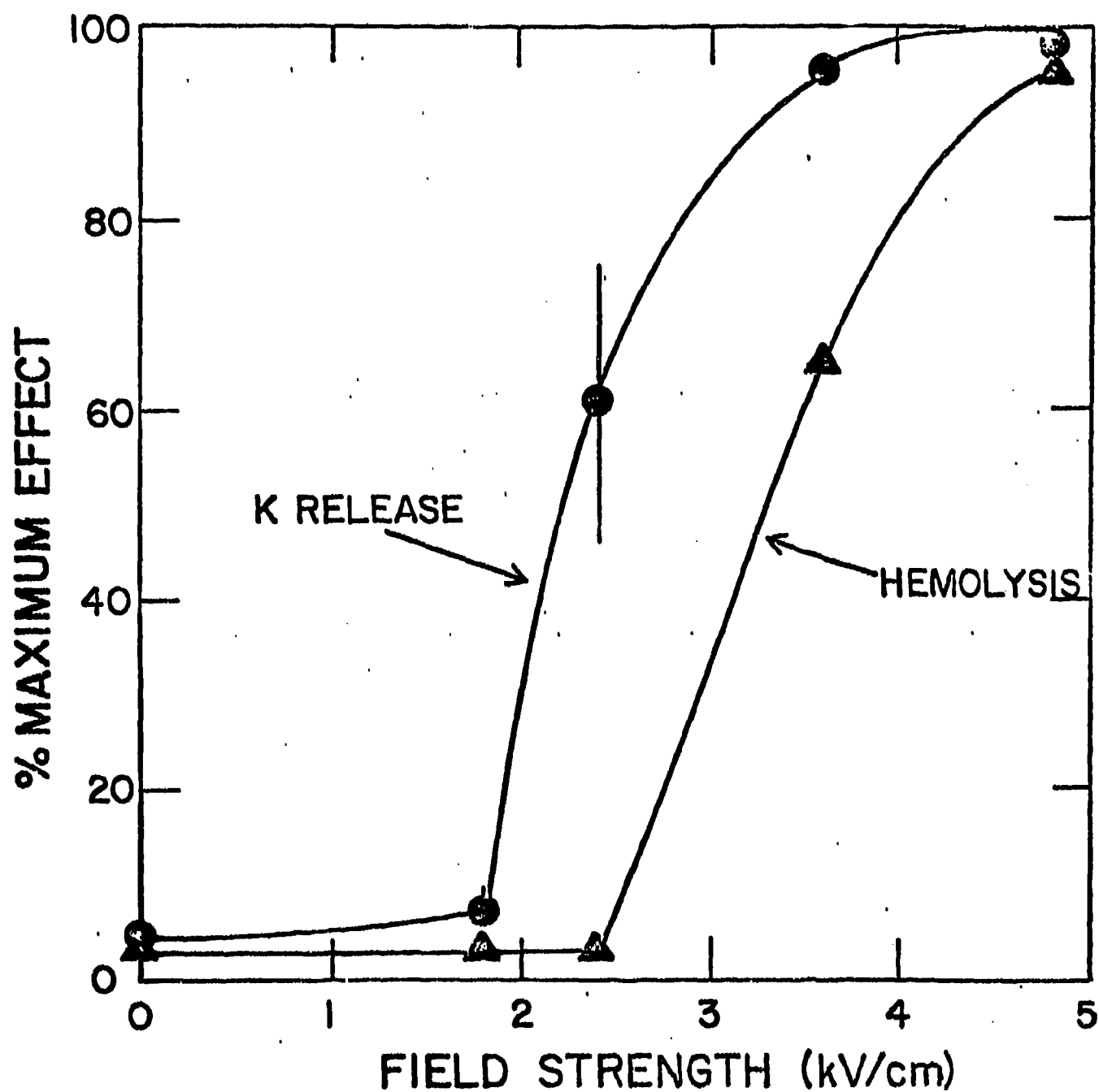


Fig. 2. Critical field strengths for potassium and hemoglobin release from human erythrocytes. The cell suspensions were exposed for 6 min to pulses with a decay time constant of 6 μ sec and with the amplitudes shown on the abscissa. The data are averages for samples from several human donors.

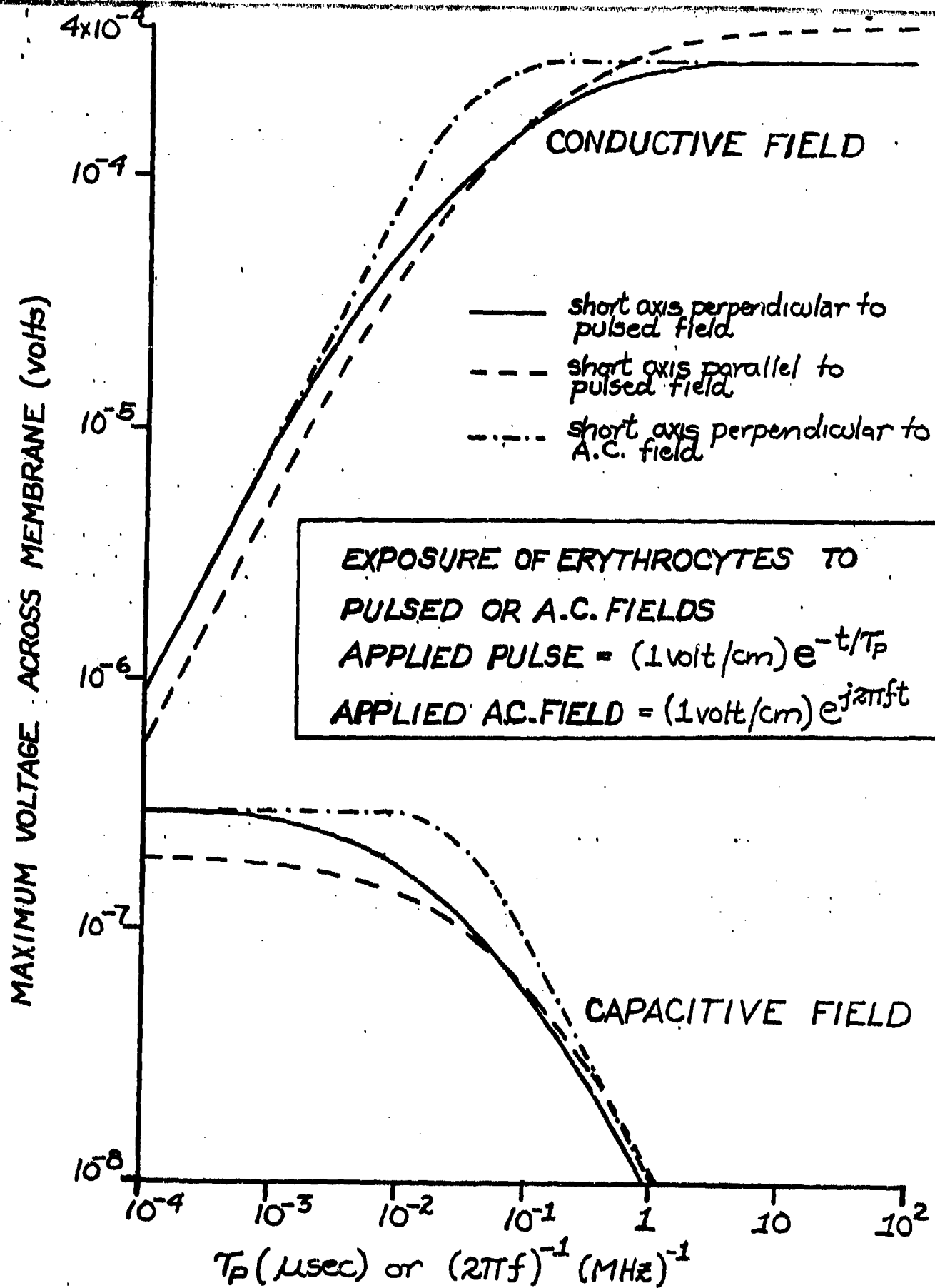


Figure 3

These results strongly support the hypothesis that transient alterations in cell membrane permeability are induced by transmembrane potentials from external electric fields, as previously suggested 1,8,10.

The time constant τ_R for the development of the transmembrane voltage is ~~0.1 μ S~~ rather than 10 μ S as reported by Riemann et al.⁷. ~~Handwritten: 0.1 μ S~~

The development of a transmembrane voltage requires charging the membrane capacitance which determines the relaxation time constant and the characteristic frequency f_0 observed in impedance measurements of cell suspensions 11-13. τ_R and f_0 are related by $2\pi\tau_R f_0 = 1$. We have observed a relaxation time constant that sets an upper limit of $\tau_R < 0.08 \mu$ S for dog erythrocytes. Impedance measurements 14,15 and theoretical calculations 10,13 also support the limit $\tau_R < 0.1 \mu$ S for the erythrocyte membrane. On the basis of the experimental results it is not possible to determine whether the dependence of the membrane alterations on the pulse decay time constant is determined by the membrane charging time constant, τ_R . However, theoretical calculations indicate that for the 0.43 μ S pulse a membrane charging time constant of greater than 0.6 μ S would be necessary to explain the observed membrane alterations. Since $\tau_R < 0.1 \mu$ S, this suggests that the time constant for membrane permeability alterations, following the establishment of a critical transmembrane potential, is on the order of hundreds of nS. *ge voir*

A mechanism for the hemolysis produced by single electric field pulses has been proposed^{3,8} in which the membrane perm-selectivity to ions is destroyed by the electric field and the hemoglobin colloid osmotic pressure causes the entry of water and subsequent swelling and bursting of the cell. Our data are not inconsistent with such a mechanism. However, in our experiments, once the membrane was sufficiently altered to allow maximal potassium release, hemolysis was produced relatively rapidly by additional pulses. Exposure to additional pulses caused hemolysis to

occur more rapidly than could be explained on the basis of a diffusion-controlled effect ⁸.

~~An alternative mechanism for the hemolysis observed~~

~~is that the initial pulsed-field exposures induce~~

~~pores in the membrane which are then favorable sites for current flow~~

~~during subsequent pulses until ultimately the pores are large enough~~

~~for the passage of hemoglobin.~~ The graded response of the erythrocyte

membrane to alterations in K^+ permeability, osmotic fragility, and hemolysis following multiple pulsed-field exposure suggests a progressive cumulative or persistent membrane alteration. The fact that exposure to fields with time constants of 0.43 μ sec or less, at a field strength of 4.8 KV/cm, which is known to produce hemolysis for longer duration pulses, did not result in hemolysis, even when the cells were exposed to thousands of pulses, ~~and the~~

~~for the step-wise growth of the membrane pores.~~ Exposure to longer duration voltage pulses, on the other hand, results in hemolysis

if the cell is exposed to a sufficiently large number of pulses; the number of pulses required being inversely proportional to the pulse duration. In this case, therefore, for hemolysis to occur there must be a summation of the effects of the individual subthreshold pulses and a lower limit for the persistence of the individual pulse effects can be estimated from the pulse period which was 10 sec in the case of the 6 μ sec pulses.

The observed relaxation times for conductance transitions in gated ionic channels in lipid bilayers are known to vary from approximately 10^{-3} to 10 sec¹⁶ which are of the same order as the persistence time of the field-induced erythrocyte membrane alterations. It has also been determined that a given channel in membranes of different lipid composition has invariant conductance and voltage-dependence but shows large variations in relaxation times ¹⁷. It may thus be suggested that the variation in the

sensitivity of erythrocytes from different species with known differences in membrane lipid composition, to multiple-pulse electric field effects is related to variation in the relaxation time of induced conductance transitions in gated channels.

~~For the induction of permeability changes in biomembranes, it is necessary to know the conditions of exposure to the electric field and to obtain information on the relationship of induced permeability changes to the conditions of exposure, pulse duration and pulse repetition rate.~~

In addition to providing information of direct application to a determination of the mechanisms of interaction of pulsed electrical fields with biomembranes, such data may be used to determine the conditions of maximum cell sensitivity to such exposure and thus be of pertinence in the evaluation of pulsed field effects in living systems.

III. IN VIVO STUDIES OF EMP EFFECTS IN THE DUTCH RABBIT

The Dutch rabbit was selected for the investigation of the effects of EMP field exposures since this species had previously been the subject of an extensive series of investigations by us of the effects of low-intensity (c.s. 5-25 mW/cm²), continuous wave and pulse modulated microwave exposure, thus providing a means of comparing the effects of these two modalities of exposure. The blood volume of the Dutch rabbit is also large enough to permit serial sampling to monitor temporal variations in serum chemistry and enzyme levels in response to EMP exposure. The sampling procedure used in these studies consisted of drawing a 5 ml blood sample from the marginal ear vein of unanesthetized rabbits ten days prior to EMP exposure which served as a baseline sample. Samples were obtained immediately pre- and post-exposure, as well as at intervals of up to two weeks post-exposure in some instances. Litter-mate Dutch rabbits 8 to 12 months old with a mean weight of 2.2 kg were randomly assigned to either the treatment (EMP) group or the sham-irradiated control group. Each group consisted of six to eight animals. Food and water was provided ad libitum except during the two hours of exposure.

The results of the effects of EMP exposure on the serum chemistry of the Dutch rabbit have been previously described in detail¹⁸. In summary, it was found that there were no statistically significant alterations in serum chemistry following 2 hours of exposure in an EMP simulator with a peak field strength of 1.5 KV/cm at a pulse repetition rate of 38 ± 2 Hz. The simulator pulse is best described as an exponentially decaying cosine waveform, the amplitude of which decreases to one-half the peak field strength in 4 cycles which at a characteristic frequency of 23.5 MHz defines a pulse duration of approximately 0.4 μ sec. The pulse rise time was less than 0.1 μ sec. The EMP simulator characteristics have been described in detail elsewhere¹⁹.

The levels of the serum enzymes alkaline phosphatase and SGOT were elevated in the immediate post-exposure samples, but the results were not statistically significantly different from the values from the sham-irradiated controls. Serum triglyceride levels were also determined in Dutch rabbits exposed under the same conditions with no apparent effect. The effects of EMP exposure at field strengths of 1.9 or 0.9 KV/cm and pulse repetition rates of 24 and 10 Hz on sodium pentobarbital-induced sleeping time in the Dutch rabbit again revealed no significant analeptic effect¹⁸.

In order to further investigate the in vivo effects of EMP exposure on rabbit serum enzymes, a series of experiments has been conducted in which the dependent variables were the ~~serum enzyme levels~~ of ~~serum enzymes~~. CPK isoenzymes are tissue specific and thus alterations in the levels of these enzymes in serum may be related to tissue specific EMP effects. The isoenzymes are denoted as MM which is found predominantly in skeletal muscle, MB which is associated with cardiac tissue, and BB which is primarily localized in brain tissue. CPK isoenzyme levels were determined by the method described by Nealon and Henderson²⁰. The exposure and sampling procedures were the same as those employed for the serum chemistry and serum triglyceride studies. Limitations imposed by the availability of the exposure facility and experimental animals dictated the need to conduct a series of CPK studies during the contracting period employing exposed and control group sizes of 4 or 5. The results of these experiments, which have been previously described¹⁸, indicated that in any given experiment the isoenzyme levels were increased following EMP exposure for 2 hours to 2 KV/cm pulses at a pulse repetition rate of 40 to 50 Hz. Statistical analyses of the differences in the mean CPK isoenzyme levels between exposed and

sham-irradiated controls revealed that although consistent elevations were induced by EMP exposure, the differences were of marginal statistical significance, whereas the overall significance of the combined responses, as evaluated by means of a sign test, was significant at the 1% level ¹⁸.

The results of these experiments were suggestive of either a treatment effect of EMP radiation resulting in a small but consistent elevation in serum levels or a systematic error or bias due to inadequate control measures. An additional experiment was therefore performed using a larger sample size and employing a different sham irradiation procedure. In previous experiments the sham irradiations consisted of placing the control animals between the plates of the EMP simulator in the same position as the exposed animals for the same exposure duration of 2 hours without energizing the pulser. In this case the sham-irradiated animals were not exposed to the pulsed field nor were they exposed to the acoustic stimulus which originated from the spark gap which discharged the pulser during EMP exposure. Another possible difference in the exposure conditions may have been the fact that ozone was generated by the spark discharge and although the EMP simulator was well ventilated, there was a possibility that the exposed animals were exposed to higher ozone concentrations than the sham-irradiated controls. To minimize the effects of such variables on the CPK response, the sham irradiation procedure was altered so that the sham-irradiated animals were placed in a mock-up of the EMP simulator placed immediately adjacent to but outside of the Faraday cage in which the EMP simulator was located. The sham irradiations were then performed simultaneously with the EMP exposures. The results of this experiment, which is referred to as Experiment 5 are summarized together with the four previous experiments (numbered 1 to 4) in Table I.

TABLE I
EFFECT OF EMP EXPOSURE ON RABBIT CPK ISOENZYMES

% Difference in Serum CPK Isoenzyme Concentrations*					
Experiment Number	Number of Animals/Group	MM Fraction	MB Fraction	BB Fraction	Total CPK
1	6	27 (0.22)**	43 (0.3)	73 (0.3)	48 (0.25)
2	6	141 (0.1)	103 (0.07)	10 (0.4)	92 (0.07)
3	4	19 (0.2)	82 (0.02)	42 (0.18)	29 (0.07)
4	3	31 (0.2)	33 (0.3)	72 (0.13)	36 (0.13)
5	6	27 (0.2)	- 0.02 (0.46)	- 43 (0.05)	9 (0.40)

* % Difference = $\frac{\text{Exposed group mean} - \text{Sham group mean}}{\text{Sham group mean}} \times 100$

** () p value for t test of differences in mean CPK values

Comparing the results of these experiments reveals that the variation in the sham irradiation procedure used in Experiment 5 reduced or reversed the positive effects detected in the previous experiments, suggesting that the CPK isoenzyme levels were affected by the operation of the spark gap trigger of the EMP simulator. Whether this was due to aural stimulation or the effect of ozone or other unknown factors is not known at present. Although CPK isoenzyme levels in serum are known to be altered as a consequence of physiological stress, there are no data on the effects of physical or chemical agents on these enzymes other than the fact that exposure to heat stress causes elevations in serum levels¹⁸.

An investigation of the effects of EMP exposure, under the conditions previously detailed, was also conducted to determine effects on serum cation concentrations. This experiment employed the same sham irradiation procedure as in Experiment 5 of the CPK determinations described previously. The mean and standard error of the mean serum sodium concentration for the group of six Dutch rabbits exposed to the EMP field was 142.4 ± 3.4 mEq/l as compared to a mean (\pm S.E.) of 138.8 ± 4.9 mEq/l for the sham irradiated controls. This difference was not statistically significant at the 5% level, nor was the difference in the mean serum potassium concentrations which were 4.3 ± 0.1 and 4.4 ± 0.2 mEq/l for the EMP exposed and sham-irradiated controls respectively.

The results of the in vivo studies of the effects of EMP exposure of the Dutch rabbit, under the conditions of this experiment, do not indicate a significant effect upon the dependent variables investigated. The in vitro studies of the effects of pulsed conductive electrical fields on erythrocyte membranes, on the other hand, have revealed significant alterations in cell membrane permeability. The threshold field strength for the induction of erythrocyte membrane permeability changes

leading to K^+ efflux was found to be 2 KV/cm and the minimum time constant for this effect for an exponentially decaying pulse was 0.4 μ sec, regardless of the number of pulses applied to the membrane. In order to compare these threshold values with the in vivo exposure conditions, the maximum induced field strength must be calculated in the experimental animals exposed to the capacitive field of the EMP simulator used in these experiments.

We have determined that the field induced in an experimental animal exposed to a time varying capacitive field is given by:

$$E_1 = \omega_0 dE_e/dt. \quad (1)$$

where E_1 is the induced field strength, ω_0 is a constant which is dependent upon the conductivity of the exposed sample and E_e is the strength of the applied electrical field¹⁸. In the present case, the EMP field E_e may be represented as a decaying exponential pulse with a rise time τ_r and a decay time τ_d . The maximum induced field in this case is:

$$E_1^{\max} = E_e^{\max} / \omega_0 \tau_r, \quad (2)$$

where $\omega_0 = 2.96 \times 10^{10}$ radians/sec. For an estimated EMP rise time (τ_r) of 0.1 μ sec and a maximum applied field strength 2 KV/cm, the maximum induced field strength at the surface of the experimental animal is on the order of 1 V/cm. Even allowing for errors in the estimated EMP rise time and the conductivity, the amplitude of the induced field is at least one order of magnitude less than the threshold determined for membrane permeability alterations. The EMP pulse duration of 0.4 μ sec is of the same magnitude as the pulse duration threshold for alteration of the K^+ permeability of the erythrocyte membrane. It may be concluded therefore that the results of the in vivo experiments are not inconsistent with the in vitro results if the in vivo biological endpoints are dependent upon field-induced physical alterations in membrane permeability. In

view of the fact that the in vitro studies have, to date, been restricted to erythrocyte membrane alterations, it is possible that other cells, such as neurons, may exhibit markedly greater sensitivities to pulsed fields which would not be reflected in alterations in the biological endpoints investigated in this study. There is an obvious need to extend the investigation of the in vitro effects of pulsed fields to other cell types in order to determine the maximum sensitivities to such exposures. Additional data on the effects of variations in the pulse parameters, namely the pulse duration, field strength, pulse repetition rate, and exposure duration are needed to more fully evaluate the potential for pulsed field alterations in living systems. Since the availability of exposure facilities for in vivo exposures is limited, such studies would most logically be performed with cell model systems.

In order to provide a basis for the comparison of the fields induced in cell membranes in response to various types of external fields we have calculated the maximum transmembrane potential in response to applied alternating currents or exponentially decaying pulsed fields for an erythrocyte oriented either parallel or perpendicular to the electric vector of applied conductive and inductive fields.

IV. ERYTHROCYTE TRANSMEMBRANE POTENTIAL

In order to relate the effects of exposure of the erythrocyte membrane to pulsed conductive electrical fields to effects of inductive fields and to harmonically varying fields it is necessary to determine the relationship of the induced transmembrane potential to cell orientation and to the parameters of the applied field, primarily to the pulse duration in the case of an exponentially decaying field or to the frequency of the harmonic field. The time dependency of the induced transmembrane field will depend upon the passive electrical characteristics of the membrane, namely the resistivity and the capacitance which determine the charging time constant or the membrane relaxation time constant (τ_r).

The charging time constant of the erythrocyte membrane is equal to the cell relaxation time constant as determined by dielectric dispersion. If it is assumed that the voltage induced in a membrane exposed to a voltage step function is exponential in time, it is possible to use Laplace transform methods to calculate the transmembrane potential for any waveform of the applied voltage. The transfer function defined as:

$$H(S) = R(S)/I(S) \quad (3)$$

defines the membrane response in the transformed domain where $I(S)$ and $R(S)$ are the transformed input and response functions corresponding to the time domain functions $i(t)$ and $r(t)$. The time domain response function may be determined from the relationship:

$$r(t) = L^{-1}(I(S)H(S)), \quad (4)$$

where L^{-1} is the inverse Laplacian transfer operator.

The maximum transmembrane potential may be related to the amplitude of an external C.C. electric field E_0 by the relationship:

$$V_m = F_j a_j E_0, \quad (5)$$

where F_j is a dimensionless factor dependent upon the cell shape and orientation with respect to the field, and a_j is the semi-major axis of

the cell parallel to the applied field¹³. The time dependency of the membrane potential in response to a step-function voltage increase E_0 is given as:

$$V_m(t) = r(t) = F_j a_j [S(1 + \tau_r S)]^{-1}. \quad (6)$$

The transmembrane potential due to an exponentially decaying applied field may be determined by using as the input function

$$i(t) = E_0 \exp(-t/\tau_p), \quad (7)$$

where τ_p is the pulse decay constant. The Laplace transform of $i(t)$ is thus:

$$I(S) = E_0 \tau_p (1 + \tau_p S)^{-1} \quad (8)$$

and the transmembrane potential generated by an externally applied exponentially decaying pulse is thus obtained by substituting Equations 6 and 8 into Equation 1 and taking the inverse Laplace transform to obtain:

$$V_m(t) = F_j a_j E_0 \left[\frac{\tau_p}{\tau_p - \tau_r} \right] \left[e^{t/\tau_p} - e^{t/\tau_r} \right] \quad (9)$$

The time at which this maximum transmembrane potential occurs (t_{\max}) which is determined by taking the time derivative of Equation 9 is:

$$t_{\max} = \tau_p \tau_r \ln(\tau_p / \tau_r) (\tau_p - \tau_r)^{-1}, \quad (10)$$

and the maximum transmembrane potential is

$$V_m^{\max} = F_j a_j E_0 (\tau_p / \tau_r)^{-1} (\tau_r / \tau_p - \tau_r)^{-1} \quad (11)$$

The capacitive field induced transmembrane potential may be determined from the conductive field transmembrane potential defined by Equations 9 or 11 by use of the relationship

$$E_i(t) = \omega_0^{-1} (dE_0/dt) \quad (12)$$

where $E_i(t)$ is the time dependent field induced by exposure to an external capacitive field $E_0(t)$. If it is assumed that $E_0(t)$ is an exponential pulse with a decay time of τ_p then,

$$E_i(t) = (\omega_0 \tau_p)^{-1} \exp(-t/\tau_p). \quad (13)$$

The maximum transmembrane potential in this case is related to the maximum conductive field transmembrane potential (V_m^{\max}) by:

$$V_{m_1}^{\max} = V_m^{\max} (\omega_o \tau_p)^{-1}, \quad (14)$$

where ω_o is a constant which depends upon the electrical characteristics of the cell.

The transmembrane potential induced by exposure to a conductive harmonic external field (i.e. A.C.) of frequency f may be determined as above to yield:

$$V_m^{\max} \text{ (A.C.)} = F_j a_j E_o \left[1 + (f/f_o)^2 \right]^{-1/2} \quad (15)$$

where f_o is the characteristic frequency of the cell system which is related to τ_r by:

$$f_o = (2\pi\tau_r)^{-1} \quad (16)$$

The maximum induced transmembrane potential generated by an alternating capacitive field may be determined by use of Equation 15:

$$V_{m_1}^{\max} \text{ (A.C.)} = 2\pi F_j a_j E_o f \omega_o^{-1} \left[1 + (f/f_o)^2 \right]^{-1/2} \quad (17)$$

In order to compare the harmonically alternating field-induced transmembrane potential with the potential generated by a pulsed field the frequency is related to the pulse duration by $f = (2\pi\tau_p)^{-1}$ and the following limiting cases may be defined as the pulse duration is allowed to approach 0 or infinity:

$$\lim_{\tau \rightarrow 0} \left[\frac{V_m^{\max} \text{ (pulse)}}{V_m^{\max} \text{ (A.C.)}} \right] = 1, \quad (18)$$

and

$$\lim_{\tau \rightarrow \infty} \left[\frac{V_m^{\max} \text{ (pulse)}}{V_m^{\max} \text{ (A.C.)}} \right] = 1 \quad (19)$$

Thus, in the limiting cases the transmembrane potentials in response to an alternating harmonic field or a pulsed field are equal.

These equations were used for an erythrocyte model to determine the relationship between the maximum transmembrane potential and the pulse duration or the frequency of an harmonically varying field for both conductive and capacitive fields. The shape and orientation parameters used in the calculations were derived from Bernhardt and Pauley¹³. In the case of an erythrocyte with the major axis oriented parallel to the applied field, $F_j = 3.06$ and $a_j = 1 \mu\text{m}$, and for the perpendicular orientation, $F_j = 1.2$ and $a_j = 3.5 \mu\text{m}$. The relaxation time constant of an erythrocyte is $\tau_r = 0.072 \mu\text{sec}$. For pulse durations longer than $0.1 \mu\text{sec}$ the perpendicular orientation results in a higher relative transmembrane potential than the parallel orientation, with the reverse being true for shorter pulse durations. A pulse duration τ_p of the same duration as the relaxation time constant of the erythrocyte (τ_r) induces a maximum transmembrane potential that is 50% of the potential induced by an equivalent alternating field.

The results of this analysis, which are shown graphically in Figure 3 indicate the marked dependency of the induced transmembrane voltage on the pulse duration. For pulse durations in the range of 1 nsec to $1 \mu\text{sec}$ the voltage induced by a capacitive field is at least one order of magnitude less than that due to a conductive field with the difference increasing markedly for pulses longer than $1 \mu\text{sec}$. A comparison of the maximum voltage induced in the membranes of various cell types (i.e. various ratios of semi-major to semi-minor axes and various orientations) in response to conductive and induced or capacitive fields has been undertaken. In all cases the conductive field induces a maximum potential under D.C. conditions, whereas the induced field maximum voltage increases as the pulse duration and pulse rise times are decreased. Although the maximum transmembrane potential is strongly

dependent upon cell type in the conductive field case, the membrane potential is to a first approximation independent of cell type for capacitive fields as shown in Figure 4.

Voltage induced across membranes of various cell types by pulsed or alternating fields. Incident external applied field amplitude 1 V/cm. Unhatched bars represent conductive field response, hatched bars represent inductive field response.

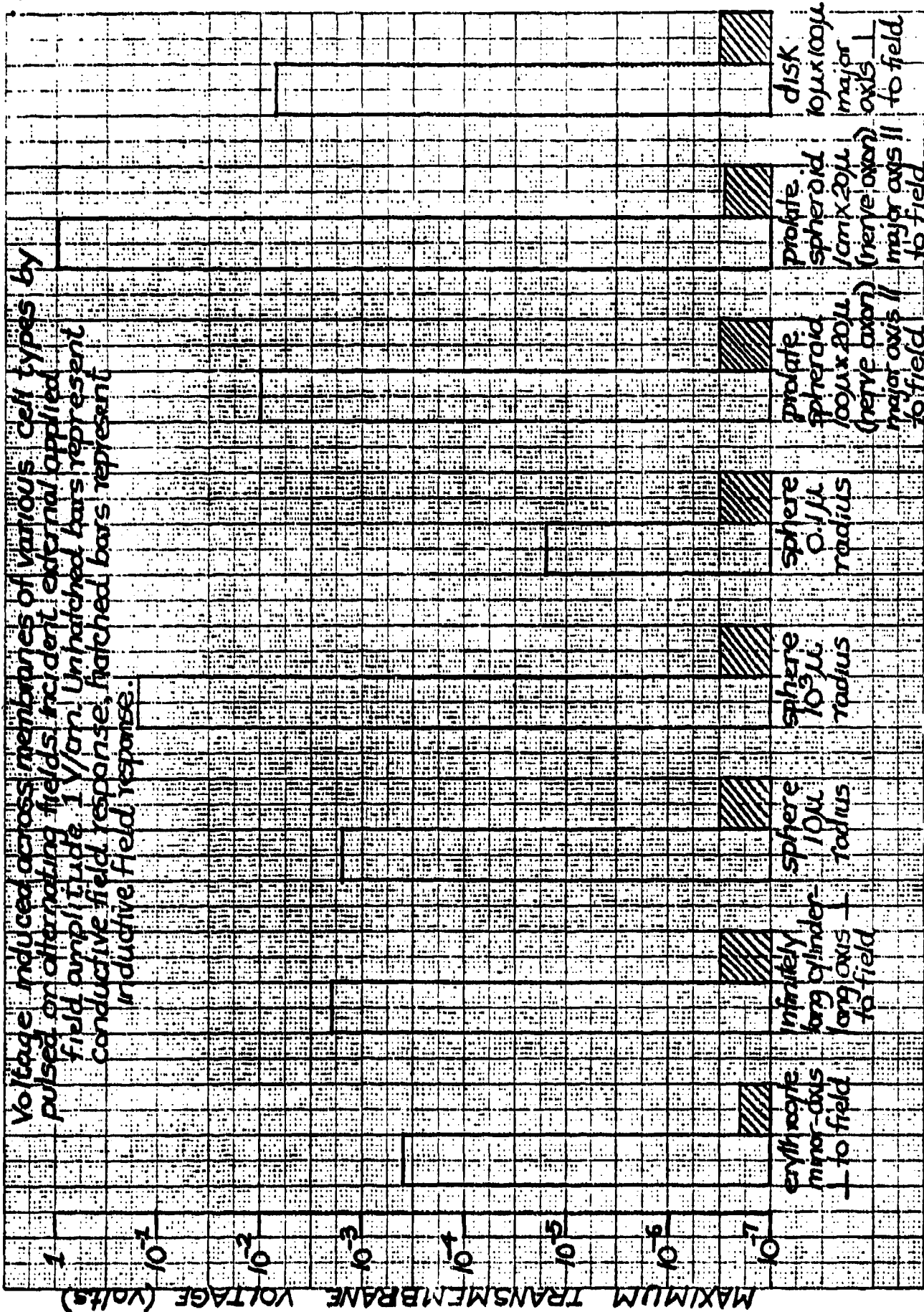


Figure 4

V. SUMMARY AND CONCLUSIONS

In vitro exposure of rabbit, dog, and human erythrocytes to pulsed electrical fields leads to alterations in the membrane permeability to K^+ , Na^+ , and hemoglobin as well as an increase in osmotic fragility. The magnitude of the response is dependent upon the species as well as the applied field strength, pulse duration, the pulse repetition rate or inter-pulse duration and the total number of pulses. A field strength threshold of 2 KV/cm and a pulse duration threshold of $>0.4 \mu\text{sec}$ has been established for K^+ release, the most sensitive dependent variable investigated. These results strongly suggest that the responses are due to the induction of transient pores or channels in the cell membrane, the sizes of which are related to the duration and strength of the applied field. Exposure of erythrocytes to multiple threshold pulses results in the same responses as above-threshold pulses, suggesting that the pulsed fields are capable of inducing transient pores, the persistence of which has been estimated to be on the order of 10 seconds or longer. These results suggest that pulsed field-induced effects in living systems would be dependent upon the magnitude of the transmembrane induced voltage as well as the pulse duration and the number of pulses.

Theoretical analyses of the induced transmembrane potential in cells of various shapes and sizes indicates a marked variation in the induced potential for conductive field exposures, as contrasted to inductive or capacitive fields which produce significantly less variation as a result of differences in cell size and shape parameters. It has been determined that there is at least a three order of magnitude greater transmembrane potential generated by a conductive field in a 1 cm long by 10 μm radius prolate spheroid (a model e.g. of a neuron) than in an erythrocyte. The same cell types exposed to a capacitive field results in a difference in

transmembrane potential of less than a factor of two. An analysis of the dependency of the induced membrane voltage on the pulse duration indicates that whereas the maximum voltage is induced by a D.C. conductive field, the maximum in the case of a capacitive field is approached as the pulse rise time is decreased to less than 1 nsec. Application of these results to a comparison of the in vitro effects of pulsed conductive fields on erythrocytes with the results obtained by in vivo exposure of Dutch rabbits to the capacitive field of an EMP simulator, provides a basis for an understanding of the absence of detectable alterations in the dependent variables investigated in the in vivo study due to the differences in the induced transmembrane potentials in these two exposure conditions. A more complete analysis of the effects of pulsed fields on living systems will require an extension of the in vitro studies to include a variety of mammalian cells other than the erythrocyte and a wider range of field parameters.

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